

THE ROLE OF COLLAGEN ON MEAT TENDERNESS IN TROPICALLY ADAPTED
CATTLE

By

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To my family

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Abstract of Thesis Presented to the Graduate School
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Factors affecting meat quality in Brahman influenced steers ($n = 53$) were evaluated to determine the underlying causes of inferior tenderness. In the first study, carcass traits, trained sensory panel scores and objective Warner-Bratzler shear (WBS) force values were collected and compared across four breed groups ranging from 0-100% Brahman. Carcasses from 100% and 50% Brahman genetics had the least amount of subcutaneous and intramuscular fat and had the lowest quality grades and cooked fat percentages ($P < 0.05$). Trained sensory panelist did not find a difference ($P > 0.05$) in meat tenderness or connective tissue amounts. However, WBS values were higher ($P < 0.05$) as Brahman influence increased. These results indicate an apparent difference in meat quality attributes between breed groups.

The second study extracted insoluble collagen from longissimus dorsi samples taken 48 h postmortem to visually assess differences in collagen content amongst the breed groups. There were no differences found ($P > 0.05$) from the most insoluble, triple-stranded cross-linked molecule to the single-stranded collagen monomer.

Results presented do not support the hypothesis that differences in meat tenderness from heavily influenced Brahman steers are the result of more extensively cross-linked collagen molecules. Outcomes from these studies indicate a strong need for more research on factors affecting tenderness and especially for Brahman influenced animals, as it remains unknown.

CHAPTER 1 BACKGROUND INFORMATION

According to Coleman (2012), nearly 30% of the United States cowherd is located in the Gulf Coast region. In this subtropical climate, cattle are exposed to harsh environmental conditions including elevated temperatures and humidity, increased exposure to parasites and resulting diseases, lower quality forages, and a more limited feed supply when compared to other regions of the country (Cartwright, 1980; Thrift, 1997; Turner, 1980). Based on this, *Bos indicus* cattle have been incorporated in breed rotations to overcome the harsh environment as well as to add reproductive longevity and maternal advantages to the herd (Brown et al., 1995; Frank, 1980; Smith et al., 2007).

Brahman is the most commonly used breed in subtropical climates based on characteristic differences in sweat glands, hair and skin properties, and thermoregulatory attributes compared to English breeds (Hansen, 2004; Smith et al., 2007). Huffman et al., (1990) noted that the ability of Brahman cattle to efficiently feed and maintain in heat-stress conditions allows them to excel and surpass *Bos Taurus* cattle finished in feed yards located in the southeast. Florida is home to 4 of the 10 largest cow-cow operations in the U.S. The majority of weaned calves shipped from Florida to large-scale feed yards contain Brahman genetics. Even with documented efficiency advantages, Brahman cattle are discounted at this stage and in the packing industry due to their notable disadvantage in meat quality and most specifically tenderness (Johnson et al., 1990).

Meat tenderness has been heavily reported as the number one driver for consumer satisfaction (Dikeman et al., 1987; Koohmaraie et al., 1995, 2002; Miller et

al., 1995; Morgan et al., 1991). Wheeler et al., (2001) reported that steaks from Brahman steers had the lowest tenderness palatability scores, compared to steaks from non-Brahman breeds. Additionally, Elzo et al., (2011) and Johnson et al., (1990) reported Warner-Bratzler shear force (WBS) values increased with increasing Brahman influence and consequently, lower tenderness ratings resulted.

Extensive research has been reported on variable tenderness of Brahman influenced cattle; however, little is known for the exact cause of this. Many researchers identified the increased calpastatin activity in Brahman influenced cattle to be the cause of tenderness issues (Shackleford et al., 1991a; Wheeler et al., 1990; Whipple et al., 1990b). More recently, though, Riley et al., (2005) evaluated many of the most important variables affecting tenderness of aged Brahman steaks including: length of sarcomere, amount and type of connective tissue, and proteolytic of z-line proteins. Insoluble collagen expressed the strongest relationship to WBS. Riley et al., (2005) called for further exploration of insoluble collagen to better understand and improve tenderness in Brahman influenced cattle.

Based on this work, it is hypothesized that a large component of variation in tenderness comes from the insoluble collagen portion and consequently, advanced mechanisms must be thoroughly researched. Steers from four breed groups ranging from 0-100% Brahman were evaluated for objective and subjective traits that had an effect on cooked meat tenderness. In addition, Longissimus dorsi muscle (LM) samples were taken at slaughter and collagen was extracted to determine the extent of cross-linking within each animal. Therefore, the objective of this study was to visually separate collagen from steers of different Brahman influence in order to compare the levels of

mature collagen cross-linking among the breed groups, hoping to gain further understanding of mechanisms responsible for decreased tenderness.

CHAPTER 2 LITERATURE REVIEW

The Role of Tenderness on Beef Quality

For many years, tenderness, flavor, and juiciness have been identified by consumers as the key components of a satisfying beef eating experience. Koochmaraie reported in 1995 that little variation exists in juiciness and flavor across different production operations; therefore, tenderness is the main driver of overall palatability and consumer satisfaction of whole-muscle beef cuts (Morgan et al., 1991). This need for a consistently tender product has been recognized by the beef industry as a top priority in improving quality (Koochmaraie, 1995, 2002). With the current state of the economy, it is of utmost importance to identify means of improving consistency in tenderness in order to please the consumer and ultimately profit the beef industry.

Factors that Effect Tenderness

Beef tenderness is an important characteristic affecting meat quality. Morgan (1991) declared it to be the most influential trait affecting overall palatability and consumer satisfaction. The challenge the beef industry faces lies in improving the consistency of whole-muscle beef products. To improve consistency, all dynamics must be analyzed to determine the most influential components. Calkins and Sullivan (2007) compiled numerous research studies to identify the many factors that have an effect on meat tenderness and the roles they play in the overall picture. Structural components remain the largest influencers on overall tenderness; however, additional factors must be accounted for as well (Weston et al., 2002).

Muscle Location & Use

Muscles are classified by their location and usage. Muscles of locomotion are those extensively used which, are located in the thoracic and pelvic limbs of animals. The persistent stress due to repeated use causes an increase in connective tissue proteins (Zinn et al., 1970). This connective tissue, which will be covered at length in subsequent sections, is responsible for the increase in movement that correlates to the decrease in tenderness. In contrast, muscles of support are located along the back of the animal, are significantly more tender due to their lack of use.

When comparing locomotive muscles in pasture-raised animals to the same muscles of those raised in confinement, variations in tenderness have been discovered. As with location, use of muscle produces more irreversible cross links in the connective tissue and is therefore tougher due to the increased movement (Gerrard et al., 1987). Animals kept in pens do not travel the same distance for food and water like an animal raised in a larger pasture would.

Breed

The effect of *Bos indicus* breeding on tenderness has been well documented (Crouse, 1987,1989; Huffman et al., 1990; Johnson et al., 1990; Koch, 1963; Riley, 2002, 2003, 2005; Whipple et al., 1990b). These studies have reported higher tenderness scores for *Bos Taurus* when compared to *Bos indicus*, which correlates to a superior tenderness rating. In tropical and subtropical climates, *Bos indicus* breeds, specifically Brahman, are extensively used in crossbreeding programs. Heat tolerance, adaptability, reproductive longevity, and maternal characteristics are few of the many advantages Brahman genetics provide in a crossbreeding program.

Riley et al., 2005 identified a set of factors and the influence each had on tenderness in the *Longissimus dorsi* of Brahman cattle. Carcass traits, sarcomere length, collagen content (total and insoluble), calpastatin activity, and lipid content were initially analyzed to determine their influence on myofibril fragmentation index (MFI) and Warner-Bratzler Shear force (WBS). Factors of significance that affected WBS included: Lean color, texture and firmness, insoluble collagen, skeletal maturity, and fat thickness. Of these, insoluble collagen expressed the strongest relationship to WBS. Still, Riley's model only accounted for 71% of variation in tenderness within the Brahman breed. With nearly 30% of total tenderness variation unaccounted for, it is important to further explore the factors affecting tenderness in Brahman cattle and to further investigate connective tissue components.

Previously discussed factors play an important role in variability of muscle tenderness. These additional elements play a more significant role when discussing variability in tenderness from *Bos indicus* influenced cattle. According to a review of numerous papers compiled by Weston et al. in 2002, structural components play the largest role in meat tenderness. Contractile proteins and connective tissue content have several functions in their contribution to cooked meat tenderness and will be further explored to determine how large their role is in the Brahman breed.

Structural Components

Actomyosin effect

Actin and myosin are filamentous, contractile proteins contained within the muscle. The sarcomere is the smallest unit of muscle contraction and is the repeating structural unit of the myofibril. This structure is responsible for the striated appearance of the muscle cell. Protein dense A-bands and less dense I-bands alternate causing the

striations. The area from Z-line, dark band intersecting the I-band, to Z-line is one sarcomere. The I-band is comprised of thin filaments while the A-band is made up of thick filaments with additional thin filaments (Goll, et al., 1984; Lonergan et al., 2010). Actin is the protein that makes up the backbone of the thin filaments. Myosin, a protein found to be the largest component of the backbone on the thick filaments, consists of a tail region and globular head. Muscle contraction takes place through the interaction of myosin and actin via the myosin head. This complex is referred to as actomyosin (Goll et al., 1984; Lonergan et al., 2010). The actomyosin bond becomes irreversible in postmortem muscle. Postmortem, the ATP supply has been depleted, and the myosin head can no longer facilitate muscle contraction, resulting in rigor bonds.

Sarcomere length

The sarcomere length or degree of contraction at rigor development plays a very large role in muscle tenderness. When ATP has been exhausted in the contractile state, the sarcomere is shorter and less tender than if rigor had occurred in a stretched muscle. In a relaxed muscle, actin and myosin filaments lay side by side. During contraction, these proteins interact and through the action of the myosin head, the sarcomere shortens. In a fully contracted muscle, the actin filaments overlap.

Mullins et al., (1969) found significant correlation in crossbred steers containing one-fourth Brahman between sarcomere length and WBS values. Through multiple studies, it is concluded that cattle with more tender beef have longer sarcomeres (Mullins et al., 1969; Weaver et al., 2008). In addition to muscle location, temperature of chill can also influence sarcomere lengths during *rigor mortis*.

Chilling Temperature

Early postmortem chilling temperatures affects sarcomere length. Lonergan et al. (2010) stated when the early postmortem chilling temperature is 0-10°C, sarcomeres could be shortened up to 50%. Additionally, he reported sarcomeres 30% of their length when held at a temperature between 20-40°C pre-rigor. Carcasses held in the range of 15-20°C experienced the lowest amount of shortened sarcomeres at 10%; however, chilling at this temperature is not practical during the pre-rigor stage. Observably, previous work on high-temperature conditions pre-rigor have a significant effect on post-mortem tenderness.

Proteolytic Enzymes

A second phase of tenderness involves tenderization, which, works to counteract the effects toughening has on the meat. Much research on this topic acknowledges that the extent of proteolytic of target proteins within the muscle fibers is responsible for a main portion of tenderness (Johnson et al., 1990; Kemp et al., 2010; Koohmaraie & Geesink, 2006; Pringle et al., 1997; Taylor & Geesink, 2001; Taylor et al., 1995a). The extent of alteration of muscle structure and proteins dictates the tenderness of meat. Numerous proteolytic enzymes have been broadly researched to determine their roles in meat quality. Of these, the calpain protease family has been found to be a significant contributor to meat tenderization (Kemp et al., 2010; Koohmaraie & Geesink, 2006; Sentandreu et al., 2002). The calpain family consists of two forms, μ -calpain, m-calpain, which require calcium for activation. Associated with this particular enzyme family is the calpain-specific inhibitor calpastatin (Kemp et al., 2010; Wendt et al., 2004).

The calpain system works by degrading proteins on or near the z-line, which is the location of the actomyosin bonds in the sarcomere. Inhibitory action of the calpastatins

requires less calcium than the calpains; therefore, calpastatins are the element of tenderness that must be targeted. It has been documented that higher levels of calpastatins are associated with lower degrees of tenderness (Kemp et al., 2010; Shackelford, 1994). Riley et al. (2005) along with (Pringle et al., 1997; Whipple et al., 1990b) discussed higher levels of calpastin activity in *Bos indicus* breeds when compared to *Bos taurus* breeds. When comparing tenderness of Brahman cattle to English and continental breeds, calpastatin activity will only explain a portion of the differences in tenderness.

Connective Tissue Proteins

Connective tissue is a very important structural component relative to muscle function. The degree to which connective tissues affect tenderness is determined by the type and amount. In order to effectively discuss the role connective tissues play in tenderness, amount, type, solubility, and cross-linking mechanisms will be discussed in detail.

Background effect on tenderness

Connective tissue is comprised of elastin, reticulin, and collagen (Marsh, 1977). Elastin is a small component in mammalian tendon, skin, muscle, and adipose tissue; however, in some tissues, including ligaments of vertebrae and arterial walls, elastin is found in more substantial amounts. This yellow connective tissue gets its name from the elastic ability to return to original shape after being stretched. Unlike collagen, elastin is not soluble during the cooking process. Reticulin is the least studied of the three connective tissue proteins. Reticulin consist of small fibers forming networks around cells, blood vessels, and epithelium. The fibers of this protein are very fine and branch out to a small degree. Of the three, elastin and reticulin are found in smaller

quantities and have shown to have very few negative affects on tenderness (Horgan et al., 1991; Jeremiah et al., 2003; Shimokomaki et al., 1972).

Collagen is contained within the muscles of all animals and it is this background collagen that is the basis for differences in tenderness. In order to recognize the effect background connective tissue proteins have on meat quality, collagen structure must first be understood.

Collagen

Collagen is the most abundant protein in mammalian animals. Collagen is found in several locations within the muscle. The epimysium is the connective tissue portion that surrounds the entire muscle and is not associated with background toughness as this can be separated easily from the muscle. Endomysial connective tissue surrounds the individual muscle fiber. Lastly, perimysium, connective tissue that encloses the muscle fiber bundles, plays the largest role in overall tenderness (Weston et al., 2002). Greater than 90% of intra-muscular connective tissue is located in the perimysium and thus it is largely responsible for variations in tenderness among individual animals and breeds (Sadowska, 1992). Nishimura et al., (1999) reported the rankings of various muscles as determined by their perimysium thickness (PT) which was very similar to the tenderness ranking of the same muscles conducted by Ramsbottom et al., (1947). Brooks and Savell (2003) more recently explored the role of PT and published analogous results concluding that their non-traditional way of predicting shear force values by means of PT was accurate and increased with aging. Additionally, emphasis was placed on the need for more research on this measurement type.

Types of collagen

Collagen is present in 19 different forms, each having a different role in biological systems (Bailey, 1998; McCormick, 1999). Collagen is also categorized into different types based on its structural makeup as fibrous, non-fibrous, fibril, or filamentous (Bailey et al., 1998).

Fibrous, self-assembling collagens are rod-like in shape and form a characteristic banding pattern that include type I, II, III, V, and XI. This quarter-staggered parallel arrangement describes the majority of collagens and specifically those associated with meat tenderness (Bailey et al., 1998; Weston et al., 2002).

Type I is largely associated with meat tenderness and is found in skin, muscle, tendons, organs, and bones. Type II collagen is the main component of cartilage. Type III, often found alongside type I, is the main component of reticular fibers. Type V collagens are found within cell membranes, hair, and placenta. Type XI is found in cartilage.

Type IV collagen is the only non-fibrous group found in muscle and is the “chicken wire” structure that is the framework of basement membranes (Bailey et al., 1998; Weston et al., 2002). This collagen forms the bases of cell membranes linking the fibrous reticular layer of the epimysium to the sarcolemma (Purslow, 2005).

Type VI and VII are filamentous collagens that form an anti-parallel alignment and are loosely arranged. These are referred to as minor types and their role in meat tenderness is unclear (Bailey et al., 1979; McCormick, 1994; Weston et al., 2002). Many collagens do not fall into specific fiber typing or networking categories but rather decorate the surface of the more significant types like type VI, which is associated with

interstitial tissues alongside type I collagens (Bailey et al., 1998; Vaughan et al., 1988). Type VII collagens are found in fibril anchoring formations.

The Collagen Structure

Tropocollagen is the basic structure of collagen. This long, thin molecule has a molecular weight of 300,000 kDa. Each tropocollagen molecule is comprised of three polypeptide subunits known as α chains. Each α -chain is a polyproline helix, which via hydrogen bonds, forms the well-known triple helical structure when all three are bound intra-molecularly. Two α_1 chains and one α_2 chain assemble to form an incredibly strong molecule (Bornstein & Piez, 1966). Type (I) and type (III), collagens of emphasis for meat tenderness, share the same repeating sequence of GLY-X-Y within the helix. In this amino acid sequence, X and Y can be any amino acid. Fibrillar collagens consist of around one-third glycine, one-quarter proline or hydroxyproline, and the remaining portion being non-helical telopeptides (McCormick, 1994). Hydroxyproline is very uncommon in proteins; therefore analysis of meat samples for its presence is a customary method of collagen quantification.

Cross-linking

Cross-linking that takes place within collagen is vital to maintaining the characteristic strength associated with the protein. Two types of cross links, each serving a separate purpose, are present in the molecule (McCormick, 1994; Shimokomak et al., 1972; Weston et al., 2002). Intra-molecular cross links are those formed within the tropocollagen molecule between α -chains. In certain circumstances, these hydrogen bonds can become covalently bonded to form irreversible intra-molecular cross links of the β component (Fennema, 1996). Furthermore, this molecule

intra-molecularly joins with an added α -chain in the helix to form the trimer collagen, which is so labeled as γ component (Fennema, 1996).

Inter-molecular cross links are responsible for the resistant tensile strength of the collagen molecule (Warriss, 2010). Inter-molecular cross links are formed through oxidative deamination of lysine and hydroxylysine residues, depending on the specific structure, via the enzyme lysyl oxidase (LOX). This results in the formation of aldehydes. Due to the arrangement of the tropocollagen molecule in the quarter-staggered fashion, the aldehyde residues react with additional aldehydes as well as lysine and hydroxylysine residues on nearby collagen molecules to form covalent bonds (Bailey, 1972, 1989; McCormick, 1994, 1999). Initially, these cross links are reducible by only having the capability of linking two molecules together (Weston et al., 2002). This fusion of molecules is reversible due to the lateral linking. Over time, cross links become more stable and fibers increase in diameter. These thermally-stable, non-reducible cross links affect tenderness not by their occurrence but rather their characteristic formations. Trivalent bonding of collagen can branch out from individual linkage with quarter-staggered molecules to transversely connecting collagens from neighboring molecules; this bonding forms a strong, three-dimensional network (Bailey, 1989; McCormick, 1999; Weston et al., 2002).

Enzymes Involvement in Cross-linking

LOX, as previously referenced, catalyses the enzymatic oxidation of lysine to an aldehyde, which, can then covalently link to an adjacent aldehyde group (Reiser, McCormick, Rucker, 1992). This highly important, cross-linking enzyme is synthesized as an inactive precursor, pro-LOX, which is triggered by the procollagen C-proteinase,

bone morphogenetic protein-1 (BMP-1) (Maruhashi et al., 2010). Cystatin-C, a protein embedded within the extracellular matrix, serves as the inhibitor for these cross-linking enzymes (Bengtsson et al., 2005). Further research of enzymatic involvement in cross-linking could be valuable in determining animals that are genetically prone to greater maturation of cross links (McCormick, 1994).

The Effect of Collagen on Meat Tenderness

The amount of collagen present in muscle tissue is important in understanding the effect on meat quality parameters; however, the type of collagen is a more direct measure of the tenderness and acceptability. When discussed, soluble and insoluble are descriptions used to identify collagen types. Stability of the collagen molecule depends on the role of cross-linking. Both immature and mature cross links form, as previously discussed, which dictate thermal stability. Intra- and inter-molecular collagen, containing a low percentage of mature stable cross links, can be broken down further during the heating process. Collagen begins denaturing around 36°C. Beginning at the ends, the triple helical structure breaks down at 64°C, which causes the molecule to shrink nearly one-fourth of its original size, resulting in muscle toughness. Additionally, heating above 70-75°C causes partial solubility and is known as gelatin (Fennema, 1996; Paul & Bailey, 2003).

Collagen left after the heating process is known as the insoluble collagen portion. Insoluble collagen is the consequence of a high percentage of mature, covalently bonded, stable cross links. The 3-D network of collagen fibers maintains its strength during cooking and the outcome is detectible differences in tenderness of the cooked meat product.

As animals age, soluble collagen content decreases and is replaced by heat-stabile, highly cross-linked molecules. Interesting research conducted by Etherington (1987) hypothesized that newly synthesized collagen dilutes older, heat-stable molecules and the result is a greater heat-labile collagen, on average. Newer research, however, warned researchers to be weary of this idea as collagen cross-linking and synthesis have a very complex relationship that is not solely explained by this dilution idea (McCormick, 1994). Presently, the understanding of this molecule in regard to age-related cross links is that as maturity increases, tenderness attributes decrease; therefore, soluble collagen has a lower impact on cooked meat tenderness (Dikeman & Tuma, 1971; Hill, 1966). Stolowski et al. (2006) reported that muscles with the highest percentage of soluble collagen had the lowest shear force values.

Type and amount of collagen were believed to be the main explanation for variations in tenderness, and as a result, several studies have been conducted over the years. Berry et al., (1974), Reagan et al., (1976) and Bailey & Light (1989) reported research in support of this theory. Opposing arguments as well have been formed based on studies by Smith & Carpenter (1970), Cross et al. (1973), and more recently Riley (2005).

As expressed, the relationship between collagen type and solubility with tenderness has been contradictory. Due to the inconsistencies, it is important for research advancements to move toward focusing on understanding the mechanisms behind the formation of mature cross links, which cause the decrease in solubility. In addition, as a subtropical region, it is critical to have the knowledge of Brahman influence as it affects the rate and amount of cross-linking. This unknown element

could explain some of the recognizable differences between tenderness in steaks derived from *Bos indicus* and *Bos Taurus* genetics.

Methods of Insoluble Collagen Quantification

Determination of insoluble collagen has been widely used over multiple decades. Research presented by Neuman (1950), Stegeman (1958), and Hill (1966) formed the basis for methods of quantifying insoluble collagen through hydroxyproline assays. The unique presence of hydroxyproline in animal proteins is a trademark of collagen (Dorfman, 1959). A certain concentration of hydroxyproline is known in collagen; therefore, the extra amounts correspond with different tenderness levels.

Most commonly used, the Hill procedure works through separation of muscle proteins: myofibrillar proteins (salt soluble), sarcoplasmic proteins (water soluble), and connective tissue proteins (acid soluble). Once heat-labile and insoluble fractions have been separated, hydroxyproline procedures are conducted for analysis. This lengthy process has been adapted a plethora of times, aiming to simplify the procedure while obtaining a more accurate measure of insoluble, soluble, and total collagen components.

This protocol has been widely used and is currently the most common method for collagen determination. The limitation to this procedure, however, is the lack of detail in this crude analysis of a highly complex system. Additionally, this method has been based upon the assumption that a known concentration of collagen is constant throughout all animal tissues. Based upon these indefinite determinants, there is a need for alternate procedures to be used for analysis in order to breakdown the individual collagen constituents for further understanding of the protein.

Current Research

The methods previously discussed have been widely used for determination of soluble, insoluble, and total collagen quantification; however, they do not explain important components within the insoluble fraction. In aims to separate and quantify levels of cross-linking, alternative methods were used in the present study.

Insoluble trimer molecules represent the highest degree of cross-linked collagen and ultimately have the greatest, negative effect on muscle tenderness. By isolating extractable, insoluble collagen portions, we may be able to determine differences in tenderness based upon the extent of cross-linking rather than the traditional methods, which only report the insoluble content amount. This can be influential in understanding not only differences in breeds but also individual animal differences within breeds.

In the present study, connective tissue components were the main focus as the most influential constituent of meat tenderness. Research conducted by Riley (2005) called for further investigation of the insoluble collagen portions of tenderness, which was based on the strongest relationships to shear force of all tenderness components analyzed. Understanding the degree of cross-linking within the insoluble collagen component could be an explanation for the known variations in tenderness.

Research from the presented data, along with cooperative studies, aim to take a deeper look at the insoluble collagen constituent of tenderness. This understanding is a positive step towards improving the beef industry through greater understanding of beef tenderness.

CHAPTER 3 EFFECT OF BRAHMAN GENETICS ON CARCASS CHARACTERISTICS, SENSORY ATTRIBUTES, WARNER-BRATZLER SHEAR FORCE, AND COOKED FAT MEASUREMENTS

Introduction

In subtropical climates, the incorporation of Brahman genetics in crossbreeding programs has become a popular commercial practice. Most desired for their heat tolerance, Brahman influenced cattle also add maternal attributes, parasitic resistance, and improved growth traits. These beneficial traits, however, are offset by inadequate meat quality attributes, of which tenderness is the most important (Johnson & Huffman, 1990, Whipple et al., 1990b). Cattle prices and carcasses are discounted as a result of the negative implications on meat quality (Crouse et al., 1989; Riley et al., 2005).

Consumers have consistently reported tenderness as the key component to a satisfying eating experience (Dikeman et al., 1987; Koochmaraie et al., 1995, 2002; Miller et al., 1995; Morgan et al., 1991). The majority of consumers are willing to pay a premium for a guaranteed tender product (Boleman et al., 1995). Variability in tenderness throughout the meat industry continues to be a highly researched topic in anticipation for a working solution. A broader understanding of factors that affect these inconsistencies is a step toward improving tenderness.

An extensive list of factors antagonistically works against producing a more uniformly tender end product. In addition to breed and genetics, myofibrillar components, type and amount of collagen, and fat deposition have all been identified as influential elements of meat tenderness (Weston et al., 2002). The objectives of this study were to compare carcass characteristics, sensory attributes, tenderness values,

and cooked fat content of fed steers from four different Brahman influenced breeding groups.

Materials and Methods

Animal Selection

Cattle in this study were part of a long-term genetics study involving Angus, Brahman, and Angus-Brahman crossbreeding. Established standards for animal care and use were followed and research protocols were approved by the University of Florida Institutional Animal Care and Use Committee (IACUC number 201003744).

Cattle in the study were assigned to four breed groups. Angus cattle were classified by having 26/32 or greater Angus genetics, Brangus cattle were those having 20/32 Angus genetics, Half Blood cattle ranged from 14-18/32 Angus genetics, and Brahman cattle were those ranging from 0-9/32 of Angus genetics. Fifty-three steers from the 2010 calving season were selected from these four breeding groups.

Reproduction, Feeding and Management

Cows were synchronized in March with an intra-vaginal progesterone device for 7 d (CIDR, Pfizer Animal Health, Hamilton, New Zealand), and subsequently injected with 5 mL of PGF_{2α} (LUTALYSE, Pfizer Animal Health, Hamilton, New Zealand) after removal of CIDR. Subsequently, cows were artificially inseminated twice, and then exposed to a natural service sire for 60 d (six single-sire natural service groups, one for each breed group of sire). Calves were born from mid-December to mid-March, males were castrated at birth, and all were weaned in September.

Cows and calves were kept on bahiagrass (*Paspalum notatum*) pastures throughout the year with free access to a complete mineral supplement (Lakeland Animal Nutrition, Lakeland, FL). Winter supplementation consisted of Bermuda grass

(*Cynodon dactylon*) hay, cottonseed meal, and molasses. After weaning, steers were taken to the University of Florida Feed Efficiency Facility (FEF) in Marianna, Florida for 100 d, and then transported to a contract feeder (Suwannee Farms, O Brien, Florida). Steers at the FEF were placed in pens and fed a concentrate diet composed of whole corn, cottonseed hulls, and a protein, vitamin, and mineral supplement (FRM, Bainbridge, Georgia, US). Steers were provided a standard commercial corn-silage diet with vitamins and minerals at the contract feeder until they reached a subcutaneous fat thickness of approximately 1.27 cm.

Carcass Data Collection

Cattle were sorted into two groups visually based on external fat thickness and were shipped to FPL Food LLC (Augusta, GA) in April and in June. Cattle were harvested under USDA, FSIS inspection. Carcasses were ribbed between the 12th and 13th ribs 24 h postmortem and HCW (kg), lean and skeletal maturity, marbling, fat thickness (FOE), Ribeye area (REA), hump height, and color score were collected. Two 2.5 cm thick steaks were removed from the 12th rib end of the whole rib from each carcass. Steaks were chilled on ice and transported to the University of Florida Meat Processing Center (Gainesville, FL) and separated into Warner-Bratzler shear force (WBS) determination and sensory analysis groups. Roughly 250mg of sample from the Longissimus dorsi muscle (LM) were removed from each WBS steak for collagen extraction. Steaks were placed in heat shrink vacuum bags (B2570; Cryovac, Duncan, SC), vacuum packaged using a Multivac C500 (Multivac, Inc.,

Kansas City, MO), and aged for 14 days postmortem at $2 \pm 3^{\circ}\text{C}$, until being frozen at -40°C prior to testing.

Warner-Bratzler Shear Force Analysis

At 24 h prior to cooking, steaks were thawed at $4 \pm 2^\circ\text{C}$. Preheated Hamilton Beach Indoor/Outdoor open top grills (Hamilton Beach Brand, Washington, NC) were used to cook steaks according to the American Meat Science Association guidelines (AMSA, 1995). Steaks were cooked to an internal temperature of 71°C , flipping once at 35°C . Thermocouples (Omega Engineering, Inc., Stamford, CT) were placed in the geometric center of each steak to constantly monitor temperature. Temperatures were recorded using 1100 Labtech Notebook for Windows 1998 (Computer Boards, Inc., Middleboro, MA). Steaks were then chilled at $4 \pm 2^\circ\text{C}$ for 24 h. After cooling, 6 cores, 1.27 cm in diameter, were removed parallel to the orientation of the muscle fibers. Each core was sheared once, perpendicular to the orientation of the muscle fibers using an Instron Universal Testing Machine (Instron Corporation, Canton, MA) with a Warner-Bratzler shear head at a speed of 200 mm/min.

Sensory Attributes

Cooked steaks were sliced and served to panelist in warmed, covered containers. Each panelist evaluated 4-6 samples, 2 cubes per sample (1.27 cm^2), in individual cubicles within a meat sensory panel room designed with positive pressure air flow, cubicles, and lighting to ensure an objective assessment. A panel of 7-11 trained members, in accordance with the AMSA sensory guidelines, assessed each sample for 5 attributes. These evaluated sensory traits included juiciness (1= extremely dry, 2= very dry, 3= moderately dry, 4= slightly dry, 5= slightly juicy, 6= moderately juicy, 7= very juicy, 8= extremely juicy), beef flavor intensity (1= extremely bland, 2= very bland, 3= moderately bland, 4= slightly bland, 5= slightly intense, 6= moderately intense, 7=

very intense, 8= extremely intense), overall tenderness (1= extremely tough, 2= very tough, 3= moderately tough, 4= slightly tough, 5= slightly tender, 6= moderately tender, 7= very tender, 8= extremely tender), connective tissue (1= abundant, 2= moderately abundant, 3= slightly abundant, 4= moderate amount, 5= slight amount, 6= traces amount, 7= practically, 8= none detected), and Off-Flavor (1= extreme Off-Flavor, 2= strong Off-Flavor, 3= moderate Off-Flavor, 4= slight Off-Flavor, 5= threshold; barely detected, 6= none detected).

Cooked Fat

Rapid determination of fat utilizing high temperature solvent extraction was conducted using ANKOM Technology's Method protocol with modifications only to reagents (hexane in place of petroleum ether). One to two cooked LM samples were placed into labeled filter bags and weight was recorded as "W1". Filter bags were heat-sealed within 4 mm of the top to encapsulate samples. Samples were placed in a drying oven for 3 h. Samples were cooled, weighed, and recorded as "W2". Samples were put in a holder and the holder was placed in the extractor for 10 min at 105°F. After extraction, samples were placed into the drying oven for 15 min. Samples were again cooled, weighed, and weights were recorded as "W3".

Crude fat was determined by the equation:

$$\% \text{ Crude Fat} = \frac{100 (W_2 - W_3)}{W_1}$$

Statistical Analysis

All data was analyzed as a completely randomized design using animal as the experimental unit. Data was analyzed using the PROC MIXED (Sas Inst., Cary, NC) procedure. Breed was the designated fixed effect and animal within breed was

considered random. The PROC MIXED procedure of SAS was used. P-value differences were obtained using the PDIFF option of the LSMEANS statement. At an $\alpha \leq 0.05$ differences were recorded as significant and tendencies were measured at an $\alpha \leq 0.10$.

Pearsons correlation coefficients were obtained for WBS and sensory tenderness to compare all factors using the PROC CORR function in SAS. At an $\alpha \leq 0.05$ differences were recorded as significant and tendencies were measured at an $\alpha \leq 0.10$.

Results and Discussion

Carcass Characteristics

Table 3-1 shows least squares means for carcass merit from the four breed groups of steers. Brangus LM was more mature colored ($P = 0.03$) than LM from the other breed groups, which did not differ. With all steers grading in the “A” maturity category and having a very weak correlation to tenderness traits, there is not a practical application to the significance of this effect. Skeletal maturity, HCW, ribeye area, yield grade, and dressing percentage did not differ ($P \geq 0.18$) between groups. Yield grade did not differ between in breed groups but the numerical trends compliment previous findings. Historically, Brahman cattle have little subcutaneous fat, which allows them to effectively survive the hot climate. Although not significant, dressing percentage trends were similar to numerous studies expressing that as Brahman influence increases, dressing percentage increases linearly (Elzo et al., 2011; Huffman et al., 1990; Koch et al., 1982; Pringle et al., 1997). Huffman et al., (1990) reported a linear increase in dressing percent from Angus steers to one-quarter Angus genetics. Elzo et al., (2011) reported the same linear trend in their data set of 1367 steers; however, dressing percentage increased from Angus all the way to Half-Bloods. Differences in these

results can be explained partially due to extreme differences in sample size (1367steers vs. 125 steers). Some earlier work credits this difference to smaller gastrointestinal tracts compared to *Bos taurus* bred cattle (Butler et al., 1956; Carpenter et al., 1961).

Subcutaneous fat thickness was significantly different ($P = 0.011$) among breeds with Brahman steers measuring the lowest at the 12th and 13th rib and Brangus steers having the most fat. Carcasses from Brahman and Half-Blood steers had less fat ($P = 0.002$) than carcasses from Brangus steers. Pringle (1997) reported carcasses from *Bos indicus* influenced cattle had less subcutaneous fat than carcasses from *Bos Taurus* cattle. Carcasses from Angus steers had the greatest degree of marbling ($P < 0.0001$) and carcasses from Brahman cattle had the lowest degree of marbling ($P = 0.02$) of the breed groups, respectively. Marbling scores determined USDA quality grades directly. Marbling scores and quality grade continue to follow the pattern discussed by all similar studies, Elzo et al., 2011, Huffman et al., 1990; Johnson et al., 1990, Pringle et al., 1997, Riley et al., 2005, to name a few. Summarizing these studies as a whole, Brahman genetics negatively affect marbling scores, which determine quality grade and carcass pricing, if paid on a quality grid. Angus steers, in this study, contained adequate intramuscular fat for the low choice quality grade while Brahman steers, on average, graded USDA Select. Neely et al., (1998) and Smith et al., (1987) designed experiments to compare USDA quality grades to consumer detectable palatability and produced agreeable results. Several concluding results declared quality grades, as a means of differentiating between LM cuts, were not as important as hypothesized.

Sensory Characteristics, WBS, Cooked Fat

Table 3-2 shows least squares means of the breed groups for objective cooked LM tenderness, trained sensory values, and cooked fat percentages. For objective WBS scores, there was a significant ($P = 0.008$) breed effect on tenderness. As the percentage of Brahman increased shear values increased in a linear fashion. Brangus steaks tended to be more tender than steaks from Half-Bloods ($P = 0.054$) which tended to have higher scores than Angus ($P = 0.052$). In agreement with numerous earlier studies (Crouse et al., 1989; Johnson et al., 1990; Koch et al., 1982; Shackelford et al., 1991a), current results indicate steaks from *Bos indicus* x *Bos taurus* are tougher than steaks from straight-bred English cattle.

Interestingly, trained sensory panel scores did not indicate a breed difference in tenderness ($P = 0.13$) or connective tissue ($P = 0.18$). Steaks from Angus steers tended ($P = 0.09$) to have higher scores, correlating to increased tenderness when compared to Half-Bloods, which had lower scores and decreased tenderness than the Brangus. However, means of LM cooked tenderness values for all four breed groups scored in the “Slightly Tender” category. All breeds did average within the acceptable WBS range (WBS < 5.5 kg) as did sensory evaluated tenderness (panel scores > 5); however, there were animals among the groups that fell out of that range. Angus and Brangus breed groups had 0% of steers with a 5.5 kg or greater shear value while Half-Bloods and Brahman groups had 7.6% and 16.7% of steers with unacceptable tenderness values (Johnson et al., 1990; Morgan et al., 1991). There is some uncertainty as to why detection of tenderness did not mimic WBS force patterns for differences between the cattle breeds; all averaging within the acceptability range may be one explanation.

For connective tissues scores, panelists tended ($P = 0.09$) to detect greater amounts of connective tissue in steaks from Brahman steers than cooked Angus steaks but the affect doesn't have a trend. This trend is analogous to the majority of prior findings concluding that as Brahman percentage increases so does the amount of detectible connective tissue. Johnson et al., (1990) reported a significant difference in connective tissue when comparing cool and warm season feeding; however, they did not detect differences when solely comparing sensory insoluble collagen across breed groups. The significant breed effect present for WBS values displayed minimal numeric differences between breeds, which could be an explanation for undetectable sensory insoluble collagen differences.

Juiciness and Beef Flavor did not differ ($P = 0.22$ and $P = 0.28$) among the four breed groups. All juiciness scores were in the "Slightly Juicy" category, while beef intensity scores were in the "Slightly Intense" beef flavor category. Koch (1982) and Whipple (1990) also reported a lack of breed effect on juiciness and flavor attributes due to variation in *Bos indicus* influence. Justifiably, Crouse (1989) and Pringle (1997) reported sensory juiciness and flavor scores that matched the marbling pattern, concluding that increasing Brahman genetics has a negative association with marbling and as a result, to juiciness and flavor attributes as well.

Off-Flavor did differ ($P = 0.02$) among the breeds still, all scored in the "Threshold Off-Flavor" category. Steaks from Angus carcasses had less detectable off-flavor ($P \leq 0.02$) than steaks from Brangus and Half-Blood carcasses. Rhee et al., (2004) designed a study individually comparing carcass muscles from 31 crossbred continental steers as well as looking at them as a whole for palatability among other traits. Results concluded

from simple correlations that beef flavor was highly positively correlated to Off-Flavor scores. The same trend can be seen, in this study, though the correlation is not as strong. Angus steers displayed the highest beef flavor scores and resulted in the greatest value in the Off-Flavor category. Brahman steers had very similar scores for both categories and results reflected the correlation Rhee et al., (2004) found.

While the detection of Off-Flavors by trained sensory panel may not statistically reflect results from previous studies (Elzo et al., 2012; Johnson et al., 1990; Pringle et al., 1997), animals in this study did average in the same “Threshold Off-Flavor” category as the three mentioned.

Objective analyses of cooked fat measurements were significantly different ($P < 0.0001$) among breeds. Steaks from Angus steers had the greatest cooked fat percentage ($P \leq 0.02$) of all breed groups and steaks from Brangus carcasses had greater cooked fat percentages ($P < 0.02$) than steaks from Brahman carcasses. Results from cooked fat analysis mimic statistical data in this study for marbling content, that as percentage Brahman increases, cooked fat percentage decreases in a linear fashion. With this trend, all groups expressing Brahman influence had a significantly lower cooked fat percentage when compared to the 0% steers (Angus). The largest difference noted was between steers displaying 50% or greater Brahman genetics. Johnson et al., (1990) reported parallel results when comparing Half-Bloods and greater Brahman influence to heavier Angus genetics. It is important to take note of the negative marbling trend as Brahman influence increases being that the majority of commercial producers in the subtropical U.S. incorporate a minimum of three-eighths Brahman into their herd for environmental survival.

Simple correlations were calculated for objective and sensory tenderness measures (Table 3-3). Brahman percentage was significantly correlated ($P = 0.0018$) to WBS without having an association to sensory tenderness. Recent work (2011) by Elzo et al., also emphasized the strong correlation between Brahman genetics and objective tenderness (WBS) in their 1367 steers differing in *Bos indicus* influence. Significant correlations did not exist for HCW, dressing percentage, lean maturity, and skeletal maturity to tenderness by trained sensory panel or shear force. Marbling, however, was significantly correlated ($P = 0.02$) to WBS with a stronger association than Riley et al., (2005) presented in 14 day aged LM steaks (-0.32 vs. -0.18). The strong negative association between marbling and breed influence indicate the significant role Brahman genetics play on meat quality. Sensory tenderness did not detect a strong correlation with marbling scores, but importantly, a negative correlation between marbling and WBS values existed in a the present study. In an Angus herd, Zhao et al., (2011) suggested that tenderness variations were associated with lipid metabolism based on a strong correlation between tender and tough grouped cattle. Well documented historical research, reviewed by Parrish (1974), indicated that this relationship between marbling and tenderness still only accounts for roughly 5% of the variation.

Fat thickness was significantly correlated ($P = 0.0154$) to tenderness while the negative correlation to shear force was not statistically significant. No differences in sensory tenderness were observed among the breeds therefore this is could be the explanation for the insignificant correlation to the objective tenderness analysis measure of WBS. As animals age, subcutaneous or external fat is deposited before intermuscular and lastly intramuscular fat (Hood, 1982). Measuring less fat at the rib,

Brahman influenced steers may not have had enough fat cover to deposit higher amounts of intramuscular fat. This could be an explanation for tenderness variations as we saw a significant negative association with marbling scores and WBS tenderness values.

Ribeye area and yield grade were not correlated to shear force, but the relationship between yield grade related to overall tenderness was significant ($P = 0.02$). Ribeye area for all breeds averaged in between 28-30 cm therefore a difference was not expected. As the fat thickness measurement weighs the most for yield grade calculation, it could explain the significant correlations between sensory detected tenderness and yield grade based on previous thoughts on fat deposition of Brahman cattle and marbling scores for an impact on tenderness.

Quality grade was correlated ($P = 0.0314$) to shear force without having a link to sensory tenderness, which was analogous to Riley et al., (2005). Warner-Bratzler shear had a clearly negative correlation ($P < .0001$) to tenderness attributes. Reiterating historical research, as WBS values increase, detected sensory tenderness scores decreases correlating to a tougher product (Morgan et al., 1991).

Sensory connective tissue was highly correlated ($P < .0001$) to tenderness and had a significant negative correlation ($P < .0001$) to shear force values. In the national beef tenderness survey, Morgan et al., (1991) credited sensory connective tissue analysis for increased WBS and decreased tenderness ratings in top sirloin steaks. While not of statistical significance, connective tissue scores for this study were parallel by Half-Bloods and Brahman cattle scoring lowest in sensory tenderness, lowest for connective tissue, and had the highest WBS values among the groups.

Off-Flavor was not correlated to any tenderness measure. Also, all collagen and cooked fat characteristics were absent of any correlations to tenderness. Reasons for significance and correlations between tenderness measures and collagen data will be discussed in detail in Chapter 4.

Conclusion

Overall, this study compliments previous research by adding strong correlations between Brahman influence and meat quality including tenderness and marbling scores. Marbling scores were lower and showed a significant negative association to WBS as did steers from the same genetics reported by Elzo et al., (2011). Sensory panel tenderness and connective tissue from Brahman cattle and Half-Bloods had lower scores than Angus mimicking Elzo et al., (2011). However, the much smaller data set (n =53 vs. n =1367) may have accounted for the insignificant results. Ultimately, Brahman steaks were the toughest through objective tenderness testing (WBS) concluding that as Brahman influence increases, desirable meat quality attributes decrease in comparison.

Table 3-1. Least squares means for carcass characteristics

Trait	Angus	Brangus	Half-Bloods	Brahman	P-Value
HCW, kg	290.6	295.2	286.7	285.2	0.81
Lean Maturity ¹	138.4 ^b	139.2 ^b	147.8 ^a	137.5 ^b	0.03
Skeletal Maturity ¹	142.3	143.1	143.5	140.8	0.74
Marbling ²	482.3 ^a	431.5 ^b	391.4 ^b	335.8 ^c	<0.0001
Fat Thickness, cm	1.13 ^{ab}	1.36 ^a	0.99 ^b	0.88 ^b	0.01
Ribeye Area, cm ²	76.7	74.2	72.9	75.9	0.57
Yield Grade	2.9	3.0	2.7	2.5	0.18
Dressing Percent	55.3	56.1	56.7	57.7	0.74

¹100 = A-maturity, 200 = B-maturity, 300 = C-maturity, 400 = D-maturity, 500 = E maturity.

²100 = Practically devoid, 200 = Traces, 300 = Slight, 400 = Small, 500 = Modest, 600 = Moderate, 700 = Slightly abundant, 800 = Moderately abundant.

^{abcd}Least squares means in the same row having different superscripts are significant at $P < 0.05$.

Table 3-2. Warner-Bratzler shear force and sensory attribute least squares means for breed groups

Characteristic	Angus	Brangus	Half-Bloods	Brahman	<i>P</i> -value
Tenderness ¹	5.5	5.7	5.1	5.2	0.12
Juiciness ²	5.1	5.6	5.4	5.2	0.22
Beef Flavor ³	5.4	5.2	5.2	5.3	0.28
Off-Flavor ⁴	5.8 ^a	5.7 ^b	5.6 ^b	5.7 ^{ab}	0.02
Connective Tissue ⁵	6.2	6.2	5.8	5.7	0.18
WBS, N	32.6 ^b	32.6 ^b	38.5 ^{ab}	41.5 ^a	0.01
Cooked Fat ⁶	7.3 ^a	5.6 ^b	4.6 ^{bc}	3.4 ^c	<0.0001

¹1 = Extremely tough, 2 = Very tough, 3 = Moderately tough, 4 = Slightly tough, 5 = Slightly tender, 6 = Moderately tender, 7 = Very tender, 8 = Extremely tender.

²1 = Extremely juicy, 2 = Very juicy, 3 = Moderately juicy, 4 = Slightly juicy, 5 = Slightly juicy, 6 = Moderately juicy, 7 = Very juicy, 8 = Extremely juicy.

³1 = Extremely bland, 2 = Very bland, 3 = Moderately bland, 4 = Slightly bland, 5 = Slightly intense, 6 = Moderately intense, 7 = Very intense, 8 = Extremely intense.

⁴1 = Extreme Off-Flavor, 2 = Strong Off-Flavor, 3 = Moderate Off-Flavor, 4 = Slight Off-Flavor, 5 = Threshold Off-Flavor, 6 = No off- flavor.

⁵1 = abundant amount, 2 = moderately abundant, 3 = slightly abundant, 4 = moderate amount, 5 = slight amount, 6 = traces amount, 7 = practically none, and 8 = none detected.

⁶Crude Fat expressed as percent

^{abc}Least squares means in the same row having different superscripts are significant at $P < 0.05$.

Table 3-3. Simple correlations of tenderness traits and Brahman influence on carcass characteristics, sensory attributes, and collagen content.

Variable	WBS	Tenderness
Brahman Percentage	.42*	-.22
HCW, kg	-.05	.02
Dressing Percent	.04	-.05
Lean Maturity	.09	.13
Skeletal Maturity	-.06	-.02
Marbling	-.32*	.22
Fat Thickness, cm	-.21	.33*
Ribeye Area, cm ²	-.03	-.24
Yield Grade	-.11	.32*
Quality Grade	-.29*	.21
WBS, N	1.0	-.54*
Juiciness	-.02	.49*
Beef Flavor	.14	-.03
Tenderness	-.54*	1.00
Connective Tissue	-.54*	.88*
Off-Flavor	-.03	.14
Gamma	-.19	.04
Beta	-.16	.01
Alpha ₂	-.10	-.01
Alpha ₁	-.10	-.03
Total	-.13	-.00
Cooked Fat	.00	.19

* $P < 0.05$

CHAPTER 4
EFFECT OF BRAHMAN GENETICS ON EXTRACTABLE INSOLUBLE COLLAGEN
AND SENSORY DETECTED CONNECTIVE TISSUE

Introduction

Collagen is the most abundant protein in mammals and serves as the structural scaffolding for skeletal muscle. This connective tissue protein is found in several locations throughout the muscle, with the perimysium being the most prevalent (Weston et al., 2002). Greater than 90% of intramuscular connective tissue is located in the perimysium and thus it is largely responsible for variations in tenderness among individual animals and breeds (Sadowska, 1992).

Collagen, a triple helical structure, is made of three α -chains bonded together for strengthening abilities. Collagen cross-linking occurs intramolecularly and intermolecularly. Within the molecule, β cross-linkages are formed through intramolecular crosslink's between 2- α chains and likewise, γ cross-linkages are formed through linking β -dimers with an additional α -monomer. Newly formed tropocollagen attaches to other tropocollagen molecules through intermolecular bonds, which over time become irreversible. Intermolecular crosslink's are responsible for the resistant tensile strength of the collagen molecule that can be recognized in the cooked meat product (Warriss, 2010). As an animal ages and greater stress is applied to muscles, crosslink's mature, which results in decreased solubility and tenderness.

Consumers have recognized tenderness as the single most important factor affecting a satisfying beef eating experience. As a result, they are willing to pay a premium for a product that can be guaranteed tender. Variability has been credited to breed effects and genetics, myofibrillar components, fat deposition, and collagen type

and amount (Weston et al., 2002). Research conducted by Riley (2005) called for further investigation of the insoluble collagen portions of tenderness, which was based on the strongest relationships to shear force out of many tenderness components that were analyzed.

Identifying animals genetically prone to greater amounts of mature cross-linking could be an important step in the direction of eliminating tenderness variation. The Hill procedure for collagen determination has been widely used and is currently the most common method utilized throughout the meat industry. The limitation to this procedure, however, is the lack of detail in this crude analysis of a highly complex system. Detection of soluble, insoluble, and total collagen can be effectively measured using this protocol; however, it does not explain important components within the insoluble fraction itself. The objective of this study was to compare subjective sensory panel connective tissue values and objective collagen cross-linking characteristics of four breed groups of Brahman influenced steers through visual separation and quantification of cross-linked chains of extractable, insoluble collagen.

Materials and Methods

Animal Selection, Reproduction, Feeding and Management, Carcass Data Collection

See Chapter 3 materials and methods.

Collagen Extraction

Collected muscle samples were placed on ice to thaw and weighed (110-270 mg). Samples were homogenized with a Pro 200 (Pro Scientific, Monroe, Ct) in 2-4 mL of a 0.1M Sodium Hydroxide (NaOH) solution, dependent upon tissue weight, then rocked for 24 h at 4°C. Samples were centrifuged at 12,000 x g for 45 min at 4°C. The NaOH

supernatant (SN) was removed and stored for future experiments. The NaOH pellet was further extracted by adding 1 mL 0.5M acetic acid and rocked for 24 h at 4°C. Previous centrifuge procedure was repeated and acetic acid SN was removed and stored for future experiments. Acetic acid pellets were weighed (mg) and pepsin was added (1mg/mL in 0.5 M acetic acid) at a volume (μ L) 5% of weight to digest samples. Samples were rocked overnight and centrifuged as treated previously. Pepsin/acetic acid SN was removed and neutralized in a 1.2:1 ratio of tris-hydroxymethylaminomethane: SN. Samples were stored at -80°C for further procedures.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE analysis was performed on the pepsin digested SN to separate mature cross-linked extractable insoluble collagen. Samples were diluted 1:4 and heated to 70°C for 10 min in NuPAGE® LDS Sample Buffer (4X), NuPAGE® Reducing Agent (10X), and deionized water. All samples were loaded at a volume of 10 μ l on 7% NuPAGE® Novex Tris-Acetate gels. Type 1 rat tail collagen (BD Cat # 354236) was extracted as above and used as the standard at a concentration of 0.5 μ g/ 10 μ L loaded. Running buffer (NuPAGE SDS 20x) was prepared (1X) and 800 mL was added to fill the buffer chamber of the XCell SureLock™ Mini-Cell. Gels were run at 150V constant for 2 h and 30 mins. Carefully, gels were removed from cassette and placed in glass containers for visual protein staining.

Silver Stain/Imaging/Quantification

Silver staining was performed using a kit (Pierce Chemical, Rockford, Illinois). Fixation for 18 h was the only modification to protocol. Following staining, gels were transferred to glass plates for imaging.

Imaging of gels was performed using Syngene G:Box technologies (G:Box Chemi-XR5 GENE Sys version 1.2.0.0, Synoptics 5.0 MP Camera, database 1.61). Automatic configurations were determined by selecting Silver Stain under the visible protein gel menu.

Statistical Analysis

Collagen and sensory data were analyzed as a completely randomized design using animal as the experimental unit. Data was analyzed using the PROC MIXED (SAS Inst., Cary, NC) procedure. Breed was the designated fixed effect and animal within breed was considered random. Subjective connective tissue was analyzed as a completely randomized design with animal as the experimental unit. The PROC MIXED procedure of SAS was used. P-value differences were obtained using the PDIFF option of the LSMEANS statement. At $\alpha \leq 0.05$ differences were recorded as significant and tendencies were measured at a $\alpha \leq 0.10$.

Pearson's correlation coefficients were obtained for WBS and sensory tenderness to compare all factors using the PROC CORR function in SAS. At a $\alpha \leq 0.05$ differences were recorded as significant and tendencies were measured at a $\alpha \leq 0.10$.

Results and Discussion

Table 4-1 shows least squares means for the four breed groups of steers. Figure 4-1 illustrates a labeled Tris-Acetate gel showing extractable insoluble collagen separation of steers from the breed groups. For objective measures of collagen, there was not a breed effect for the highest cross linked collagen form, γ -gamma, ($P = 0.668$) and likewise did not follow a trending pattern. β -Beta cross linked collagen also did not show any differences among breeds ($P = 0.556$). Similarly, α_2 -alpha II and α_1 -alpha I,

collagen monomers, did not show a breed effect ($P = 0.793$ and $P = 0.688$, respectively). Total extractable collagen, following the pattern, did not display any significant differences by breed ($P = 0.699$). For subjective collagen content, trained sensory panel did not indicate a breed difference for connective tissue ($P = 0.186$); however, panelist tended to detect greater amounts of connective tissue in steaks from Brahman steers than cooked Angus steaks ($P = 0.09$).

Based on responses from factors affecting tenderness, simple correlations were calculated for objective and subjective tenderness measures (Table 3-3). There were no significant correlations between any of the collagen traits and those used for tenderness measurements. Sensory detectable connective tissue, however, was highly correlated ($P < .0001$) to both WBS and sensory detectable tenderness differences.

When comparing all collagen variables, it is interesting that Brahman steers displayed the lowest insoluble content. Based on sensory analysis and WBS values for these animals, one would expect more drastic differences. Brahman cattle in this study had the lowest subjective connective tissue scores, correlating to the most detected among the groups as well as the highest values for shear force measurements. If tenderness differences in Brahman cattle is a result of the complexity of the connective tissue system rather the amount of insoluble collagen, then Brahman cattle should exhibit the greatest quantities of highly cross linked collagen bands through separation. Insoluble collagen quantities for Brahman steers were expected to be the highest among the breeds for the most highly cross linked form of collagen, gamma. This was not the case. As crosslink's mature, they become less heat soluble and develop irreversible crosslink's that cannot be broken down, even when heat is added. Sensory

connective tissue and objective WBS values are both measures of collagen that did not solubilize during the cooking process. Brahman steers scored the lowest in both categories, which would infer, through the understanding of cross-linking mechanisms, that they should possess more highly cross linked, irreversible collagen molecules compared to the heat-labile molecules associated with more tender meat products. Conversely, cross-linking data did not follow this pattern, implying there is an important underlying mechanism responsible for tenderness differences in Brahman influenced cattle that has not been explored thus far.

Brahman cattle are known for their late maturation, which could help explain a portion of the contradictory results. These purebred Brahman and crossbred animals may not have reached full maturation; therefore, higher forms collagen crosslink's may have not fully developed at the time of slaughter. Additionally, it could be hypothesized that the protein turnover occurring for longer periods of time in Brahman cattle could dilute the proportion of mature crosslink's.

Collagen content reported in this study is the result of adaptations to a standard protocol. In the final step of the process, supernatant removed from the insoluble pellet was used for analysis of each sample. This process resulted in quantification of extractable, insoluble collagen rather than a total insoluble collagen amount. While this deviation was necessary for visual representation of cross-linking patterns, it may not be a true depiction of the insoluble collagen portion. The insoluble pellet may have been the true representation of collagen that cannot be extracted or broken down any further. This then might be a better portrayal of cross-linking that correlates with higher shear force values and greater detection of connective tissue from panelists.

Conclusion

Brahman influenced cattle have a confirmed difference for objective and subjective tenderness measures. Insoluble collagen content accounts for a significant portion of the variation in tenderness therefore further understanding is necessary to improve meat quality (Riley et al., 2005). Through visual separation of cross linked collagen bands, the advanced mechanism behind collagen maturation is currently still undetermined for a correlation to meat tenderness. It would be beneficial to further explore the systems controlling irreversible crosslink's in Brahman cattle, as their unique genetic qualities may provide an explanation for inferior tenderness.

Table 4-1. Least squares means for objective and subjective collagen content

Collagen Traits	Angus	Brangus	Half-Bloods	Brahman	<i>P</i> -Value
γ^1	0.28	0.30	0.29	0.23	0.66
β^1	0.30	0.34	0.35	0.25	0.55
α_2^1	0.46	0.54	0.56	0.44	0.79
α_1^1	0.29	0.33	0.35	0.27	0.68
$\gamma+\beta+\alpha_2+\alpha_1^1$	1.34	1.53	1.57	1.22	0.69
Connective Tissue ²	6.20	6.23	5.84	5.73	0.18

¹= $\mu\text{g/mL}$

²1 = abundant amount, 2 = moderately abundant, 3 = slightly abundant, 4 = moderate amount, 5 = slight amount, 6 = traces amount, 7 = practically none, and 8 = none detected.

Least squares means in the same row having different superscripts are significant at $P < 0.05$.

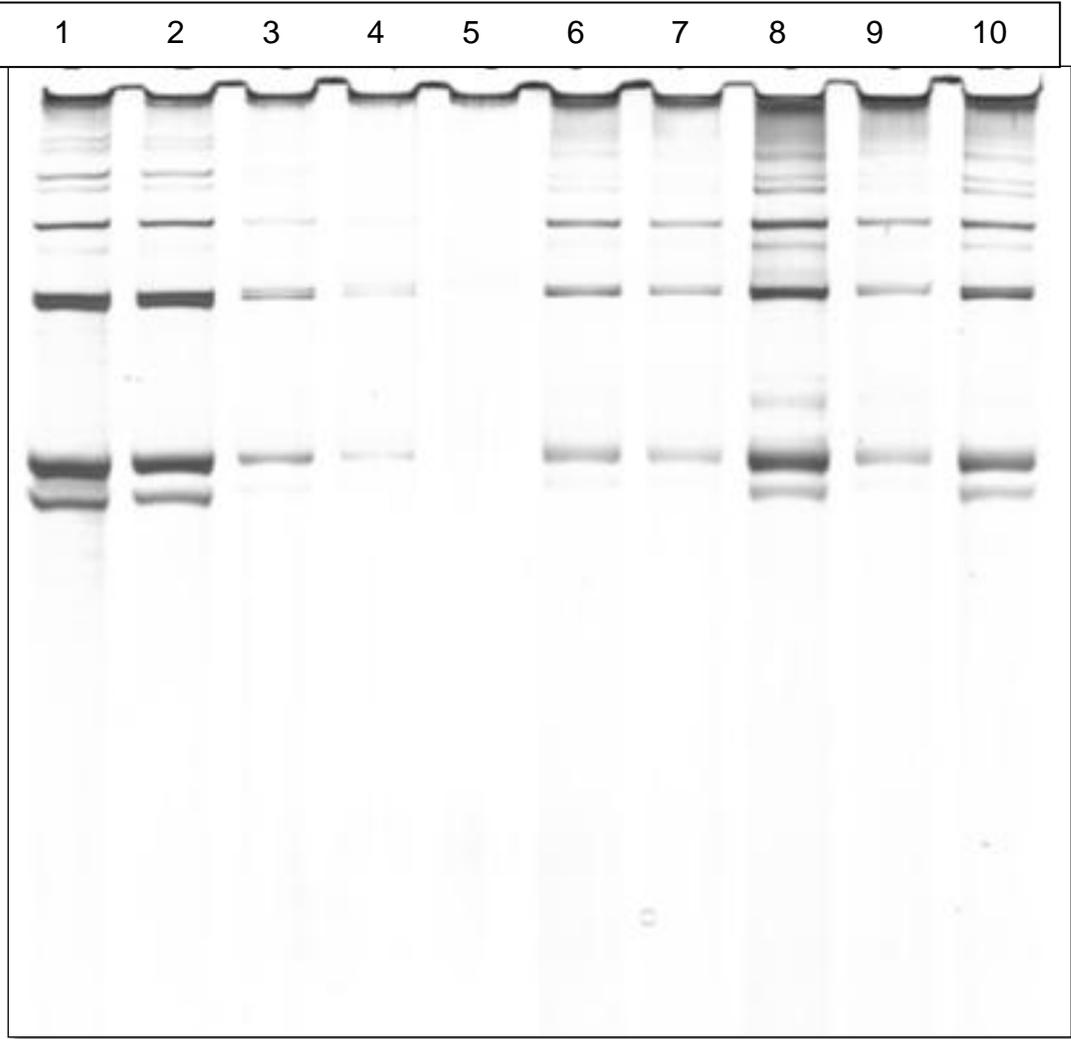


Figure 4-1. Silver stained Nu-PAGE Tris-Acetate 7-10% gel of purified rat tail collagen and extractable insoluble collagen from steer LD muscle samples from four breed groups. 1=Rat tail collagen 100 $\mu\text{g/mL}$, 2= Rat tail collagen 50 $\mu\text{g/mL}$, 3= Rat tail collagen 10 $\mu\text{g/mL}$, 4= Rat tail collagen 5 $\mu\text{g/mL}$, 5= Rat tail collagen 1 $\mu\text{g/mL}$, 6= Angus steer , 7=Brahman steer , 8=Brangus steer, 9=Half Blood steer, 10=Half Blood steer

CHAPTER 5 OVERALL IMPLICATIONS AND CONCLUSIONS

For the comparison study, results illustrated numerous differences between Brahman influenced carcasses when compared to those of Angus genetics. For carcass characteristics, lean maturity and fat thickness were found to be lower in Brahman cattle than the Angus steers utilized. More importantly, however, marbling scores, quality grade, and crude fat were significantly lower in Brahman steers, which directly affects meat quality and emphasizes the problem faced by the meat industry.

Through sensory evaluation, there was not a significant difference found between breeds for the focal trait, tenderness, nor was juiciness or beef flavor scores noteworthy. Surprisingly, significant differences were detected for the Off-Flavor category, yet these did not display a specific pattern. Connective tissue scores were lowest for Brahman steers, which indicate the greatest background toughness, but similarities in this category among groups resulted in insignificant results. Brahman cattle had significantly higher WBS values, and therefore had a cooked product that was the toughest amongst the breed groups.

Visual separation of collagen and cross-linking patterns did not show any significant differences among the four breed groups. Moreover, no trends were found in any level of collagen in correlation with Brahman genetics. Through visual separation of cross-linked collagen bands, the advanced mechanism behind collagen maturation is currently still undetermined for a correlation to meat tenderness.

Carcass and sensory data conclude major differences in meat quality of the evaluated animals. Contrary to the hypothesis, however, collagen cross-linking data did not correspond with those results. With insoluble collagen content accounting for a

significant portion of the variation in tenderness (Riley et al., 2005), a greater understanding is still necessary to improve meat quality.

This research allowed us to explore collagen on a more in-depth level than previous studies; however, results may have been more favorable had there been adaptations in the extraction protocol. It would be advisable for future work to measure the total, soluble, and insoluble collagen content via hydroxyproline assays for comparison reasons. Also, when extracting insoluble collagen, measuring the insoluble pellet would be a more accurate evaluation of total insoluble collagen versus the extractable insoluble collagen that this study quantified. In closing, it would be beneficial to explore other sources of variation in beef quality of Brahman cattle, as their unique genetic qualities may provide an explanation for inferior tenderness.

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BIOGRAPHICAL SKETCH

Marisa White was born in 1988 to Nancy and Robert White. She grew up with three sisters and one brother in Inglis, FL. She did not grow up in an agricultural family; however, after high school she began working for her local livestock auction and became very involved in the industry. Marisa completed her associate's degree in Agriculture from Central Florida Community College and then moved to Gainesville, FL to attend the University of Florida. While in UF's animal science department, Marisa was a member of intercollegiate meat judging team in 2009 and coached the team in 2010. After graduating with a Bachelor of Science degree in Animal Science, she enrolled as a master's student in the department under the direction of Dr. Dwain Johnson. During her studies, Marisa continued to coach the judging team, assist in teaching class, and helped with numerous 4-H/ FFA extension projects. In 2011, she put all her focus on her research, which identified collagen's role in beef tenderness. After graduation in August 2012, Marisa will take a job with Boars Head in Sarasota where she will be a professional member of the meat science industry.